Lack of efficacy of low dose oral interferon alfa in symptomatic HIV-1 infection: a randomised, double blind, placebo controlled trial

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Background: Interferon alfa (IFN-α) exhibits dose related in vitro activity against human immunodeficiency virus (HIV), with complete inhibition of HIV replication at IFN-α concentrations $\geq$ 256 IU/ml. In mid-1990, Kenyan investigators reported that oral administration of an extremely low dose (150 IU/day) of natural human (nHu) IFN-α resulted in complete alleviation of AIDS related complex and AIDS symptoms and resolution of opportunistic infections without additional treatment. Moreover, loss of HIV antibody seropositivity was reported in approximately 10% of treated patients. Subsequent small studies failed to substantiate these spectacular claims, but controversy on the efficacy of this treatment persisted.

Methods: We studied 559 adult Ugandan patients with WHO stage 2–4 HIV infection and a Karnofsky performance score of more than 50, who had not received any drugs with antiretroviral activity in the previous 3 months. The patients were randomly assigned in a double blind fashion either to 150 IU oral nHuIFN-α/day or placebo. The duration of treatment was extended from 28 weeks to 60 weeks 9 months after enrolment had started. At that time 112 subjects had already received 28 weeks of treatment and been discontinued from the study.

Results: Both study groups were comparable with respect to all baseline characteristics studied, except that the nHuIFN-α group had slightly lower absolute CD4+ lymphocyte counts (median 60.7×10^6/l) than the placebo group (median 85.3×10^6/l) (p=0.033). Therefore, all analyses were adjusted for CD4+ lymphocyte counts at entry. In both treatment groups there was relentless progression of HIV disease. Subjects treated with nHuIFN-α and placebo had similar mortality, disease progression rates, decline of CD4+ lymphocyte counts and Karnofsky performance scores, and prevalence of symptoms. No patient reverted to HIV-l seronegative antibody status. Serious adverse events were not seen. Quality control of the study medication documented that the active drug indeed contained IFN-α activity.

Conclusions: The current large, randomised, double blind, placebo controlled study did not show any benefit from oral treatment with 150 IU nHuIFN-α/day in a population of African patients with symptomatic HIV infection.

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Keywords: HIV infection; AIDS; interferon alfa

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Introduction

Interferon alfa (IFN-α) exhibits dose related in vitro activity against human immunodeficiency virus (HIV), with complete inhibition of HIV replication at IFN-α concentrations $\geq$256 IU/ml even in chronically infected cell lines.[1–6] In vitro the 90% inhibitory concentrations with concomitant administration of zidovudine and IFN-α were 0.02 µmol and 32 IU/ml, respectively.[7]

Treatment of patients with AIDS related Kaposi's sarcoma with high dose ($27–36 \times 10^6$ IU/day) subcutaneously administered IFN-α has resulted in tumour responses, rises in peripheral blood CD4+ lymphocyte counts, and significant decreases in serum p24 antigen levels, particularly in those patients with initial CD4+ cell counts above 50×10^6/l.[8 9] A randomised, double blind, placebo controlled trial, comparing placebo, 3×10^6 IU and 36×10^6 IU of IFN-α intramuscularly or subcutaneously three times a week, in a population of AIDS patients without Kaposi's sarcoma and a median CD4+ cell count of approximately $50 \times$ 10^6/l, showed no clear benefits for either dose of IFN-α therapy.[10] In a randomised, double blind, placebo controlled study, in patients with asymptomatic HIV infection who had CD4+ cell counts of $\geq 400 \times 10^6$/l, IFN-α 35×10^6 IU/day subcutaneously, showed clear antiretroviral effects.[11] In asymptomatic HIV infected subjects, decreases of serum p24 antigen levels have been attained with IFN-α dosages as low as 1.5×10^6 IU/day, subcutaneously.[12]

In mid-1990, Kenyan investigators reported that oral treatment with an extremely low dose (150 IU/day) of nHuIFN-α (called Kemron by the investigators) of patients with AIDS or AIDS related complex (ARC), resulted within 10 weeks in complete alleviation of symptoms commonly associated with ARC and AIDS and resolution of opportunistic infections without additional treatment in all patients. Loss of HIV antibody seropositivity ("serodeconversion") was reported to occur in approximately 10% of the patients.[13] Subsequent small studies failed to substantiate these spectacular claims.[14–16]

In September 1990, the World Health Organisation (WHO) called a meeting of researchers, representatives of national drug regulatory agencies, and pharmaceutical manufacturers to examine the conflicting evidence on the efficacy of low dose oral IFN-α. The data presented at this meeting were not conclusive and the expert committee recommended that WHO should commission a randomised double blind placebo controlled study of sufficient sample size to establish whether low dose oral IFN-α was efficacious in the treatment of symptomatic HIV infection.[17] In this paper we report the findings of this study.

Methods
PATIENT CHARACTERISTICS
The study was conducted in the Department of Medicine at Makerere University Medical School, Mulago Hospital in Kampala, Uganda. The majority of patients recruited into the study came from either the AIDS clinic of Mulago hospital or The AIDS Support Organisation (TASO). Drugs on the WHO list of essential drugs were provided for the management of infections occurring during the trial period.

Men and non-pregnant women at least 15 years of age were eligible to enter the study if they met the following criteria: had HIV-1 infection documented by enzyme linked immunosorbent assay (ELISA) and confirmed by a western blot; were in stage 2–4 of the WHO clinical staging system for HIV infection[18]; had an absolute peripheral blood CD4+ lymphocyte count of less than 300×10^6/l on two occasions more than 1 week apart; had a Karnofsky performance score of 50 or more; had serum urea, creatinine, and aspartate aminotransaminase (ASAT) levels of less than five times the upper limit of normal; gave written informed consent to participate in the study; and lived within 10 miles of the study site. Subjects were excluded if they were taking or had been taking any drugs with known antiretroviral activity in the previous 3 months.

STUDY DESIGN
This was a randomised, double blind, placebo controlled study. Subjects were given a unique incremental study number at enrolment, corresponding to their individual prepacked and prelabelled supply of study drugs, which could either be active drug or placebo, according to the randomisation code generated by the WHO. Each treatment arm initially consisted of 200 subjects, which was later revised to 280 on the recommendation of the Data and Safety Monitoring Board (DSMB) following a preplanned interim analysis in February 1992.

TREATMENT REGIMEN
The study drugs were tablets containing either 150 IU nHuIFN-α (Hayashibara Biochemical Laboratories, Okayama, Japan) stabilised in an anhydrous polysaccharide carrier, or matching placebo, to be taken daily for 28 weeks (extended to 60 weeks after a protocol amendment in February 1992). Patients were in-structed to place the tablet in the mouth and to allow it to dissolve in the saliva for 2–3 minutes, massaging gently with the tongue to facilitate dissolution and effective mucosal contact. They were encouraged to retain the saliva in the mouth for at least 30 minutes before swallowing it.

CRITERIA FOR RESPONSE
Primary measures of outcome were death, deterioration of health severe enough to warrant withdrawal from the study, and time to more advanced WHO clinical stage. Secondary measures of outcome were changes in body weight, subjective symptoms, Karnofsky performance scores, CD4+ lymphocyte counts, and adverse events. Loss of anti-HIV seropositivity was assessed at week 12.

SUBJECT TREATMENT AND EVALUATION
The pretreatment evaluation included a medical history, a physical examination, assessment of HIV serostatus, peripheral blood CD4+ lymphocyte counts, a full blood count (FBC, using a Coulter counter model T540), and serum urea, creatinine, and ASAT levels by direct analysis methods.

The baseline CD4+ cell count was taken to be the average of three CD4+ lymphocyte count readings, which were obtained 2 weeks and 1 week before enrolment and at study entry. CD4+ lymphocyte counts were done using an adaptation of the immunoalkaline phosphatase method for blood smear.[19] The slides were prepared and stained within 1 hour of collecting the blood and stored at −20°C for reading at a later time.

HIV serology was done using a competitive enzyme assay (Wellcozyme HIV-1, Wellcome Diagnostics, Dartford, Kent) and confirmed by a western blot (Diagnostic Biotechnology HIV-l BLOT, Singapore) according to the WHO guidelines.[20]

After entry into the study, the subjects were seen every 2 weeks for 28 weeks. After the interim analysis in February 1992, the protocol was amended such that treatment was extended to 60 weeks. Patients were seen every 4 weeks from weeks 28 to 60.

On each visit symptoms and signs of HIV disease progression, incident HIV or AIDS related conditions, and incident adverse events were recorded. FBC and CD4+ lymphocyte counts were done every 2 weeks up to 12 weeks, at 18 and 24 weeks, and every 4 weeks up to 60 weeks. HIV serology was repeated after 12 weeks of treatment. Patients who failed to attend scheduled follow up visits were visited at home by the counsellor or one of the study doctors to ascertain the reason for defaulting, for on site clinical assessment, and to facilitate attendance at the study clinic.

QUALITY CONTROL OF STUDY MEDICATION
The IFN-α activity of the study medication was assessed at production, and blind tested twice during and at the end of the trial on a random sample of vials, by Hayashibara Biochemical Laboratories, at Fujisaki Institute, Okayama, Japan, and by the National Cancer Institute,

Table 1 Baseline characteristics on admission

Variable	nHuIFN-α (n=279)	Placebo (n=280)
Mean age (range)	31.9 (17–67)	31.2 18–59)
Sex: male (%)	129 (46.2)	123 (43.9)
Cough (%)	132 (47.3)	156 (55.7)
Itching (%)	188 (67.4)	181 (64.6)
Blurred vision (%)	33 (11.8)	38 (13.6)
Gastrointestinal symptoms (%)	165 (59.1)	149 (53.2)
Joint or muscle pain (%)	124 (44.4)	127 (45.4)
General symptoms (%)	199 (71.3)	199 (71.1)
Mean body weight (range)	51.8 (32.0–85.5)	51.7 (31.0–85.0)
Mean Karnofsky score (range)	82.666 (50–90)	82.8 (20–90)
Mean haemoglobin (g/l) (range)	10.9 (5.4–18.0)	11.0 (6.1–17.5)
Median absolute CD4+ lymphocyte count (range)	60.7 (0.0–577)*	85.3 (0.0–562)*
Median percentage CD4+ lymphocytes (range)	3.9 (0.0–27.7)**	4.8 (0.0–27.2)**
WHO clinical stage		
Stage 2 (%)	29 (10.4)	25 (8.9)
Stage 3 (%)	112 (40.1)	124 (44.3)
Stage 4 (%)	138 (49.5)	131 (46.8)

General symptoms = tiredness, fever, or night sweats.
Gastrointestinal symptoms = loss of appetite, pain on swallowing, nausea, vomiting, or diarrhoea.
*p = 0.033; **p = 0.065.

Frederick Cancer Research and Development Centre, Frederick, Maryland, USA. Ten lozenges were sampled from each vial and assayed in duplicate, as described previously.[21]

STATISTICAL ANALYSIS
The two groups were compared on admission using the χ^2 test, the t test, or the Mann–Whitney U test. Life table methods were used to analyse survival rates and disease progression. Survival rates were adjusted for a difference in baseline CD4+ lymphocyte counts using proportional hazards regression. Logistic regression was used to analyse symptoms on follow up, while repeated measures analysis of variance was used to analyse log transformed CD4+ lymphocyte counts, body weight, and Karnofsky scores.

Patients who discontinued from the study were retained in the analyses of CD4+ lymphocyte counts, body weight, and Karnof-

sky scores after discontinuation. Their CD4+ lymphocyte counts and body weight were assumed to remain the same as at their last follow up visit; Karnofsky scores were treated in the same way unless the patient died, when they were set to 0.

Results

STUDY POPULATION
Between 10 June 1991 and 6 July 1992, 560 subjects were enrolled in the study. One subject was found after admission to have been HIV 1 seronegative at entry and was excluded from the data analysis. Two hundred and seventy nine subjects received nHuIFN-α and 280 placebo.

Table 1 shows the baseline characteristics of the population at study entry. There were no significant differences between the two groups at the time of entry with respect to age, sex, WHO clinical stage, body weight, Karnofsky performance score, symptoms, haemoglobin, or biochemistry results (not shown). Although the WHO clinical stage at admission did not differ significantly between the two groups, nine subjects in the nHuIFN-α group had Kaposi's sarcoma at entry, compared with two in the placebo group (p<0.05).

The nHuIFN-α group had lower absolute CD4+ lymphocyte counts (median 60.7 × 10^6/l) than the placebo group (median 85.3 × 10^6/l) (p=0.033). However, the CD4+ percentage values did not differ between groups at the 5% level of significance (p=0.065).

FOLLOW UP AND STUDY DISCONTINUATION
The original treatment period was 28 weeks. A preplanned interim analysis, conducted in February 1992, showed a divergence between the two groups' survival rates which, however, was not statistically significant. The DSMB therefore recommended extending treatment to 60 weeks. By this time, 112 subjects had already completed 28 weeks' treatment and been discontinued from the trial (51 from the nHuIFN-α group, and 61 from the placebo group). The study was closed on 15 January 1993, 30 weeks after enrolment of the last subject. At the end of the study 23 subjects (nine in the nHuIFN-α group and 14 in the placebo group) had completed the 60 weeks of treatment, while 116 (57 in the nHuIFN-α and 59 in the placebo group) had completed between 30 and 60 weeks. The mean and median lengths of treatment for the nHuIFN-α group were 6.0 and 6.4 months, while the placebo group they were 6.3 and 6.5 months, respectively.

One hundred and seventeen subjects left the study for reasons other than death. The reasons for discontinuation are summarised in figure 1, and were similarly distributed in both treatment groups. Forty eight subjects discontinued the study because their condition deteriorated. They either became too sick to take the study medication or were transferred to their villages where it was not possible to continue supplying them with the study drug. Twenty nine subjects withdrew their consent, including 13 who felt
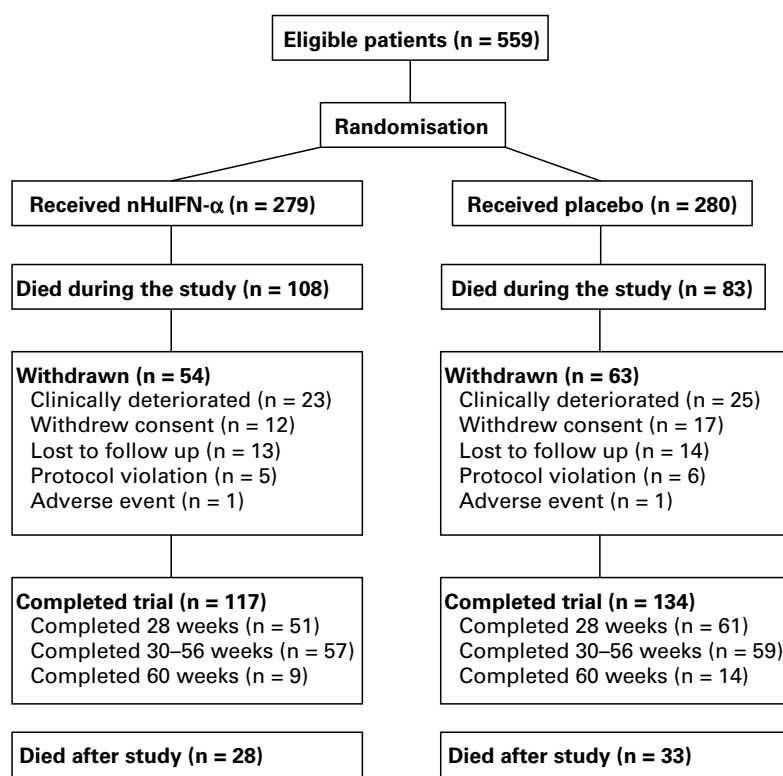


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          ┌─────────────────────────┐
          │ Eligible patients (n = 559) │
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          │     Randomisation       │
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Received nHuIFN-α (n = 279)	Received placebo (n = 280)
Died during the study (n = 108)	**Died during the study (n = 83)**
Withdrawn (n = 54)	**Withdrawn (n = 63)**
Clinically deteriorated (n = 23)	Clinically deteriorated (n = 25)
Withdrew consent (n = 12)	Withdrew consent (n = 17)
Lost to follow up (n = 13)	Lost to follow up (n = 14)
Protocol violation (n = 5)	Protocol violation (n = 6)
Adverse event (n = 1)	Adverse event (n = 1)
Completed trial (n = 117)	**Completed trial (n = 134)**
Completed 28 weeks (n = 51)	Completed 28 weeks (n = 61)
Completed 30–56 weeks (n = 57)	Completed 30–56 weeks (n = 59)
Completed 60 weeks (n = 9)	Completed 60 weeks (n = 14)
Died after study (n = 28)	**Died after study (n = 33)**

Figure 1 Flow chart describing progress of patients through the trial.
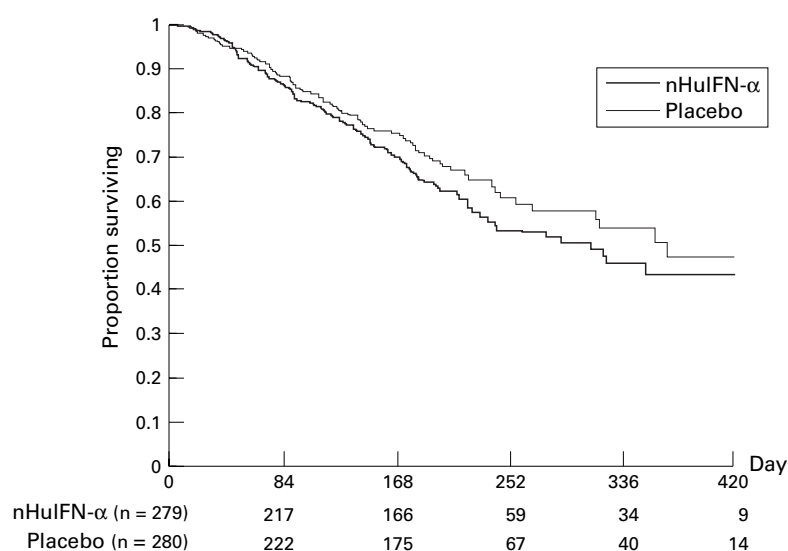
Figure 2   Survival by treatment group (adjusted for CD4+ lymphocyte count at entry).

they were not getting the improvement they had expected and one subject who withdrew from the study because she became pregnant.

Twenty seven subjects were lost to follow up and could not be traced. Eleven people violated the protocol because they missed two consecutive visits. Two subjects discontinued the study owing to symptoms which were perceived as adverse effects related to the study drug.

EFFICACY ASSESSMENT
Death
A total of 191 patients, 108 in the nHuIFN-α and 83 in the placebo group, died either during the period when they were on treatment or within 30 days of terminating the study. The unadjusted survival rate among subjects given nHuIFN-α was significantly lower than among placebo treated subjects (p = 0.035); after adjustment for the difference in CD4+ lymphocyte counts at entry, however, the survival rates in the two groups were similar (p >0.3) (fig 2).

When the study was designed, there was no intention to record information about deaths which occurred more than 30 days after stopping treatment for other reasons. However, after the study the patients remaining in Kampala were followed up by one of the investigators (ETK in his routine HIV clinic, and additional information on their vital status was available from this source). In addition to the 191 patients who died while on study treatment or within 30 days of stopping treatment, the investigator was aware of another 61 deaths (28 in the nHuIFN-α group, and 33 in the placebo group) more than 30 days after treatment was stopped. In the nHuIFN-α group, 11 of these patients had discontinued from the study because their condition deteriorated, two had withdrawn consent, and 15 had completed 28 weeks of treatment in the study's first phase. In the placebo group, seven patients had discontinued because their condition deteriorated, four had withdrawn consent, four were discontinued because of a protocol violation, one had discontinued because of an adverse event, and 17 had completed 28 weeks of treatment in the

study's first phase. When these deaths were included in analysis, there were no significant differences in the survival rates between the two groups, either with or without adjustment for the difference in CD4+ lymphocyte counts at entry.

It was not possible to establish accurately all the causes of death because of insufficient diagnostic facilities. Nevertheless, categories of causes of death (gastrointestinal, respiratory and central nervous system diseases, Kaposi's sarcoma, others, and unknown) were similarly distributed in both treatment groups. After the interim analysis in February 1992, the DSMB recommended that deaths should be recorded as HIV related or not. This information was collected for the last 99 deaths. Only one death in the placebo group, was not clearly HIV related: the patient committed suicide.

Disease progression
Rates of progression to a more advanced WHO clinical stage, clinical deterioration, or death did not differ between study groups, either before or after adjustment for the difference in CD4+ lymphocyte count at entry (both p >0.3) (fig 3A).

Disease progression rates also did not differ between groups when patients were stratified by WHO clinical stage at enrolment (all p >0.2) (figs 3B, C, D).

Change in symptoms
There were no differences between the two treatment groups at any time with respect to the prevalence of symptoms. At least 95% of the nHuIFN-α group and 93% of the placebo group patients were symptomatic at each follow up visit. The prevalence of general symptoms (tiredness, fever, and night sweats) varied between 68% and 78% in the nHuIFN-α group and between 65% to 71% in the placebo group. The prevalence of gastrointestinal symptoms (loss of appetite, pain on swallowing, nausea, vomiting, and diarrhoea) varied between 59% and 70% in the nHuIFN-α group and between 53% and 64% in the placebo group.

Changes in CD4+ lymphocyte counts
The difference in CD4+ lymphocyte counts, observed at baseline, persisted throughout the study. During treatment there was a similar steady decline in the CD4+ lymphocyte cell counts in the two groups (table 2). When the CD4+ lymphocyte counts during treatment were adjusted for the count at baseline, there were no significant differences in the CD4+ lymphocyte counts between study groups.

Changes in Karnofsky performance score and body weight
Karnofsky performance scores decreased uniformly in both groups throughout the study period: at weeks 0, 12, 24, 36, 48, and 60 the mean Karnofsky scores of subjects in the nHuIFN-α group were 83, 71, 58, 50, 48, and 45, respectively, and in the placebo group 83, 74, 63, 57, 53, and 52 respectively.
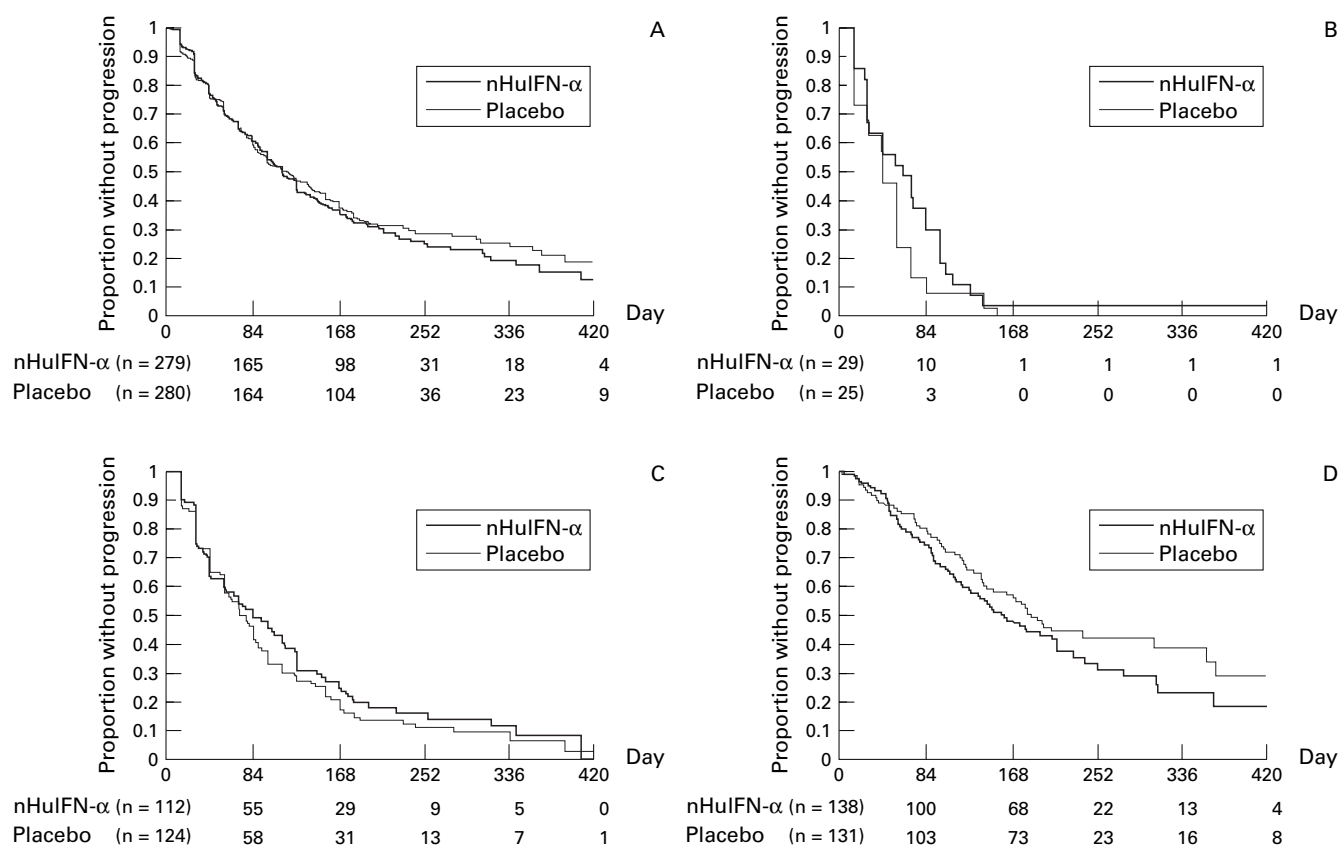
Figure 3 Disease progression (more advanced stage, deterioration, or death) by treatment group (adjusted for CD4+ lymphocyte count at entry). (A) All randomised patients. (B) Patients with WHO stage 2 disease on admission. (C) Patients with WHO stage 3 HIV disease on admission. (D) Patients with WHO stage 4 HIV disease on admission.

There was no significant change in body weight in the two study groups. Mean body weight varied from 52 to 55 kg in the nHuIFN-α group, and from 52 to 58 kg in the placebo group.

HIV serodeconversion
No patient reverted to HIV-1 seronegative antibody status in the two groups.

ADVERSE EVENTS
One subject in the nHuIFN-α group developed symptoms of chest pain, dizziness, and abdominal pain from the day she started taking the study medication. These symptoms continued for 3 days until she stopped the medication. She reported similar symptoms on restarting the medication 2 days later and was advised to stop the treatment. One subject in the placebo group had been taking the drug for 5 weeks when she developed diarrhoea which she associated with the study drug. The diarrhoea stopped when she stopped the medication a week later. There were no other discontinuations from the study as a result of adverse events.

Table 2 Median absolute CD+ lymphocyte counts during follow up (range)

Week	nHuIFN-α	Placebo
Baseline	60.7 (0–577)	85.3 (0–562)
12	26.0 (0–960)	42.0 (0–748)
24	20.0 (0–655)	46.5 (0–966)
36	18.0 (0–725)	37.0 (0–1014)
48	15.0 (0–670)	32.0 (0–1144)
60	15.0 (0–670)	32.0 (0–782)

QUALITY CONTROL OF STUDY MEDICATION
The IFN-α activity of verum and placebo tablets was assessed on 10 vials of each at production, on six verum and three placebo vials in October 1991, on three vials of each in April 1992, and on six verum and five placebo vials in September 1992. The mean IFN-α activity of verum tablets was 160 (SD 8) IU, 154 (12) IU, 145 (6) IU, and 135 (11) IU, respectively. None of the placebo tablets exhibited IFN-α activity. Independent analysis in a laboratory in the United States confirmed that verum tablets had IFN-α activity.

Discussion
The current large, randomised, double blind, placebo controlled study did not show any benefit from oral treatment with low dose nHuIFN-α in a population of African patients with symptomatic HIV infection. Except for a small difference in survival, which was slightly worse in the nHuIFN-α group, there were no differences in disease progression, symptomatology, or any of the other efficacy parameters between treatment groups. CD4+ lymphocyte counts declined relentlessly in both groups. When the analyses of survival and disease progression were adjusted for CD4+ lymphocyte counts at entry, the difference in survival between both study groups was no longer significant and no new significant differences emerged. No loss of HIV antibody seropositivity was observed.

It may be argued that the patients in this study were too severely immunocompromised to benefit from IFN-α therapy.[8–11]

Nevertheless, the results are in striking contrast with those from Kenya,[13] in a population with similarly advanced HIV infection in which an identical formulation, dose, and route of drug administration of IFN-α was used. Moreover, the present study, patients who at entry had only minor symptoms of HIV infection (WHO clinical stage 2), also showed similar disease progression rates in both treatment groups.

Interferons are generally not believed to be orally bioavailable, and are rapidly denatured upon contact with gastric secretions since they are proteins.[22] To avoid gastric denaturing, patients were instructed to keep the tablets in the mouth as long as possible in order to achieve maximum absorption within the mouth through mucosal contact. However, no known oral IFN-α receptor has been isolated to date and therefore no one knows how much of the orally taken dose is actually absorbed.[22] Even if there were substantial oral absorption of nHuIFN-α, it is hard to imagine that a daily dose of 150 IU would exert any noticeable effect on HIV replication. In vitro IFN-α concentrations of $\geqslant 4$ IU/ml are required to achieve some inhibition of HIV replication,[1] and even when a synergistic combination of zidovudine and IFN-α is used, the 90% inhibitory concentration for IFN-α is 32 IU/ml.[7] Thus it is also highly unlikely that patients at earlier stages of HIV infection will benefit from treatment with low dose oral IFN-α. Further efforts to evaluate the effect of low dose oral IFN-α therapy in patients with HIV infection would be better redirected to more rational approaches to the treatment of this deadly infection.

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